



**Supplementary Figure 2. Concentration independence of the FRET ratio in cells expressing free sstFRET.** (A) Normalized line scans of donor channel, acceptor channel and FRET ratio image. Red and black trace shows nearly 10 fold variation of fluorescence intensity across the cell in donor and acceptor channels. The green traces indicate that the FRET ratio is flat across the cell. (B) Images of cell expressing free sstFRET. Donor channel, cyan, Left panel and the middle panel, acceptor in yellow and FRET ratio image, right panel. Red lines show the line scan over the images. Scale bar, 10  $\mu\text{m}$ .